

## Progress in modern crop science research in wheat biology

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Every crop breeding effort seeks to enhance production. Despite several advances, most worldwide breeding efforts have long sought to improve grain production potential, insect/pest/disease resistance, grain quality, and stress tolerance. Almost all wheat breeding programs aim to increase grain yield potential. Wheat breeders have achieved substantial improvements in crop yield. Genetic transformation, cloning, and genetic engineering increase production potential in wheat. The primary breeding strategy for wheat is the pedigree. However, hybrids and population improvement are also utilized. Breeders utilized biotechnology to increase breeding success. Biotechnology and genome editing are examples of current technology that can improve global agriculture production by assisting crop development. Traditional wheat breeding methods have been supplemented with biotechnology to speed up wheat improvement efforts. These methods will speed up wheat biology research and help to develop wheat breeding plans. However, many programs in developing countries (especially) are still trying to include them.

**Keywords:** Wheat (*Triticum aestivum* L.), Conventional wheat breeding, mutagenesis, mutant, molecular marker-assisted breeding, genome editing technologies, genetic transformation.

### INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important staple food crop that feeds 30% of the global population. (Altaf *et al.*, 2021). The world's population is anticipated to reach 9 billion by 2050, needing considerable increases in wheat production (Altaf *et al.*, 2021; Foley *et al.*, 2011). The quality of wheat grain must also be enhanced. However, traditional breeding methods have reached their threshold in improving grain production and quality. So, a different wheat breeding approach is necessary. Rational design-based molecular breeding uses the genetic underpinning of agronomically valuable characteristics (Qian *et al.*, 2016). Researchers have created numerous elite lines using various characteristic combinations (Li *et al.*, 2013). Finding genes that control key agronomic traits is the first step in utilizing this method to create new wheat cultivars. Due to wheat's complicated and enormous genome, our capacity to undertake functional genomic analysis is confined to rice or maize.

Although this is the case, wheat's draft and near-complete genome assemblies (Jia *et al.*, 2018) have been produced. In 2016, the FAO reported harvesting 756 million tons of wheat from 220 million acres of arable land ([www.fao.org/faostat](http://www.fao.org/faostat)). Wheat also lags behind maize and rice in yield and usage of genetic technology (Uauy *et al.*, 2017). With more significant irrigation, fertilizer usage, and improved breeding, the average worldwide wheat production nearly quadrupled during the Green Revolution (Evenson *et al.*, 2003). To feed the 9 billion people (predicted by 2050), wheat yields must grow by over 60% while preserving or improving nutritional value. To achieve this goal without increasing cultivated land area, essential plant production and environmental adaptability traits must be prioritized. The historical production ceiling must be broken via molecular-based breeding and genetic engineering. A lack of this vital crop might jeopardize world food security.

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**Origin and importance:** Bread, also known as ordinary wheat, is an undoubtedly significant domesticated plant; it also has a long history in agriculture (Bilgic *et al.*, 2016). Wheat has become an essential element of the global diet, supplying vital nutrients such as protein, vitamins, and minerals. It is cultivated from 67°N to 45°S but is less frequent in tropical climates (Shrawat *et al.*, 2018). In 2016, over 749 million tons of wheat were harvested on 220 million hectares globally, ranking second among cereal crops in production and first in the harvested area. The remaining 5% comprises durum wheat (*T. turgidum* L.) and a few other less important types (Qu *et al.*, 2015).

**The origin of the species:** AABBDD genomes, ( $2n = 6 = 42$ , AABBDD genomes) produced by merging three similar diploid genomes (Krenek *et al.*, 2015). The genomes of A (*T. urartu*) and B (*Aegilops speltoides*) were split around 7 million years ago. Hybridization and homoploid speciation generated the D genome donor (*Ae. tauschii*) 5.5 million years ago. Emmer wheat (*T. turgidum*) is an allotetraploid with AABB genomes. Finally, between 8000 and 10,000 years ago, in the Fertile Crescent, *T. turgidum* and *Ae.* hybridized in Northern Iran. *T. aestivum*, allohexaploid wheat, generated *tauschii* (Ueki *et al.*, 2013).

**Approaches used in wheat biology:** Modern biology has advanced quickly by cloning the gene responsible for a mutant trait and utilizing transformation to confirm the candidate gene. Other model species approaches have not been widely utilized in wheat research due to the size and complexity of the wheat genome and issues with genetic transformation. As a result, we only have a rudimentary understanding of wheat biology. Thanks to recent advances in genome sequencing and assembly, gene cloning, and wheat transformation, experts in other fields should use the same research tools as wheat scientists.

**Mutagenesis, mutant preparation and characterization:** Mutants are vital for studying gene function and improving crop genetics. The importance of natural mutants in functional genomics and molecular breeding has been well established (Song *et al.*, 1995), and introduced spontaneous mutations of the 'green revolution gene' increased world wheat yields. On the other hand, higher plants have just 105-108 occurrences per base pair each generation (Gu *et al.*, 2005). Random mutations have been created in plants using chemical and physical mutagenesis methods. The resultant mutant populations are screened for interesting phenotypes, and mutant characterization identifies the gene responsible. Notably, these mutations can be used to enhance crops. For seed mutagenesis, EMS is a widely used chemical, and it produces many randomly distributed mutations (G to A or C to T) (Jiang *et al.*, 2010). To screen and characterize mutants, this research employed EMS-mutagenized populations of different wheat types. EMS-based mutagenesis is fast, large-scale, non-transgenic, and non-reversible.

Ionizing particles like rapid neutrons or gamma rays cause strand breakage in seeds, and cosmic radiation can also affect seed delivery. Large DNA deletions and chromosomal rearrangements ranging from one to millions of base pairs can be seen in these mutants (Wang *et al.*, 2017). Finding the genome's mutations has resulted in new plant species with enhanced traits. Use these mutagenesis methods to generate mutants that may be used to differentiate genotype-phenotype relationships. Due to the random nature of mutagenesis, large-scale screening is necessary.

Also, being an allohexaploid, wheat has three versions of most genes. In the three sub-genomes, functional redundancy produces no phenotypic response to a single homolog mutation. However, genome editing techniques, particularly CRISPR/CRISPR-associated Cas's systems, have lately allowed specific alterations in wheat (Li *et al.*, 2019). They will be crucial in identifying wheat gene functions. However, using the CRISPR/Cas system requires knowledge of the targeted gene's sequence. These two approaches for creating wheat mutants should be complementary.

**Wheat breeding:** Most wheat breeding priorities should not change. However, faster development and information collecting from different areas should need new approaches and pathways. In order to increase wheat production potential, photosynthetic capacity must be increased. The addition of C4 carbon fixing genes gives theoretical evidence to enhance it (Wang *et al.*, 2018). Gluten-rich wheat grain is known to be harmful to celiac and non-celiac gluten-sensitive people [34, 35]. Unless we can grow grain without this disadvantage, we may see a decline in wheat consumption. Fortunately, some wheat includes gluten and a chemically different form that people living with celiac disease can ingest, making it an essential future wheat breeding target (Rosell *et al.*, 2014).

**Gene cloning methods genome editing:** Genome editing is a potential new technology and plant breeding development. This technique may accurately target nucleotide deletion, insertion, or replacement (Bortesi *et al.*, 2015). Despite its vast, redundant, polyploid genome, some attempts have succeeded. A CRISPR/Cas9 method for this species has already been developed. The simultaneous alteration of three homeo-alleles of the same gene is one of the intriguing results of this method. Gene editing can also introduce the genes of wild relatives into wheat, decreasing linkage drag (Wang *et al.*, 2014).

**Genetic transgenics:** Transgenics are still controversial in our society, and it is not universally accepted owing to cultural or spiritual reasons. The scientific data has not been adequate to eliminate health concerns (Hillbeck *et al.*, 2015). There are very few records of usage of T-wheat cultivars, even 27 years after the first one, so comparisons with crops like wheat or soybeans are impossible. Some writers call wheat the "GM-abandoned cereal." Many of these traits have been studied, including resistance to powdery mildew, leaf spot, and

fusarium head blight. It has been shown to improve baking characteristics and drought resistance (Li *et al.*, 2015).

**Male sterility:** In hybrid seed production, male sterility is a valuable feature. Despite discovering five Ms loci, only Ms1, Ms2, and Ms5 have been cloned and described in wheat. Ms1 is a recessive gene that two different groups have cloned. The A and D sub-genomes, on the other hand, are epigenetically silenced. This protein is required to synthesize pollen exine and male fertility (Vendruscolo *et al.*, 2007). Microsporocytes contain it. Because it is male sterile but has no other negative agronomic characteristics, this mutant is often utilized in China to create new wheat cultivars. Ms2 was molecularly characterized in 2017. Ms2, an orphan protein, is activated by a TRIM insertion. It is present mainly in Ms2 anthers and induces dominant male sterility. Ms5 was cloned as well. For pollen exine synthesis and male fertility, a PPID-anchored lipid transfer protein is required (Ni *et al.*, 2017).

**Genetic mapping based on sequencing:** Mutational mapping (MutMap), mutant chromosome sequencing (MutChromSeq), and targeted chromosomal-based cloning via long-range assembly have all emerged as innovative and faster gene cloning techniques. MutRenSeq was created to overcome the limitations of traditional gene cloning techniques. Because genes are evolutionarily conserved, they may have identical sequences and functions in different species (Xia *et al.*, 2017). Wheat genes were discovered using homology-based cloning, which compares known and target genes' sequences. To quickly isolate genes, MutMap uses whole-genome sequencing, mutagenesis, and gene mapping. Pick a cool phenotype and cross it to a wild-type parent, then self it on MutMap. These characteristics will be present in the F2 offspring. Whole-genome sequencing is conducted on bulk DNA from F2 mutant progeny and mapped to the reference genome. SNPs with SNP index 1 are thought to contain the mutant gene (Pallotta *et al.*, 2019). This technique has been used to discover rice genes that affect important agronomic traits. With a short and high-quality reference genome, MutMap is the best choice.

**Genetic transformation of wheat:** Transforming wild-type genes into mutant plants should be used to verify the role of a candidate gene in the observed phenotype. The two most common techniques for introducing foreign genes into plant cells are biolistic transformation and Agrobacterium-mediated genetic transformation (Xia *et al.*, 2012). Conversion of plants is a massive advantage in gene function studies. The difficulty of altering and regenerating wheat has slowed progress in genetic engineering, primary research, and applied genetics. It was created by microprojectile blasting an embryogenic callus. It was later described. However, the procedure's efficiency was poor. Since then, improvements in DNA delivery techniques, target tissues, culture medium components, and selectable marker genes have increased wheat transformation efficiency (Shrawat *et al.*, 2018). Agrobacterium-based methods are preferred for

transformation because they are less genotype-dependent and more efficient (approximately 10 percent). However, the host genome has multiple transgenic copies, and the vector backbone is infrequently incorporated into the genome. The outcomes are the low copy number, complete integration of foreign genes into the host genome, and long-term transgenic expression. The Japan Tobacco Company has discovered a technique for producing transgenic wheat seedlings from immature Fielder embryos that is both efficient and effective. More cultivars were effectively treated with modest modifications (Richardson *et al.*, 2014).

**Marker-assisted selection (mas):** Breeders use marker-assisted selection (MAS) to improve genetic gain. To differentiate breeding lines in MAS, molecular markers closely linked to or co-segregate with the target trait might be employed (Collard *et al.*, 2007). MAS practitioners report genetic increases in double phenotypic selection. It may be used for single gene introgression, backcrossing into germplasm, and disease resistance gene pyramiding. MAS has also been used to pick many characteristics concurrently. While MAS has revolutionized plant breeding and increased genetic gain, it is not without flaws. MAS is less successful when screening for complex or multi-genic quantitative characteristics (Hospital *et al.*, 2009). To find novel QTLs linked with a trait, biparental mapping populations must be created. They rarely account for a complete breeding effort's allelic diversity and genetic background. Their effects vary according to the environment, notably quantitatively. Alternative mapping populations and breeding programs must be created to validate novel QTL location and allelic effects, which is costly and time-intensive (Heffner *et al.*, 2009). A possible alternative to biparental analysis might be genome-wide association studies (GWAS), which employ linkage disequilibrium to discover marker-trait correlations across a wide variety of germplasm. Assume a breeder utilizes MAS to incorporate a minor impact QTL into their breeding program before testing it with local germplasm. As a result, they may achieve more genetic progress than phenotypic selection. Employing MAS in a breeding program can be prohibitively expensive (William *et al.*, 2007). Generalized selection (G.S.) complements genetic mapping methods since it addresses the genetic architecture of complex characteristics.

**Wheat yield and quality-related genes:** Discovering and describing genes that impact grain production and quality is essential for rational design-based molecular breeding of new wheat varieties. Tall wheat stalks cannot support heavy grains. To improve yields, dwarfing or semi-dwarfing genes must be introduced into wheat. Wheat's Rht gene has been identified. Its codes for a gibberellin-signaling transcription factor with an SH2-like domain. New Rht-mutated varieties have aided in the Green Revolution (which response abnormally to gibberellin). Wheat thousand-grain weight increases by the gw2-A1 mutant gene, making grains thicker

and longer (Simmonds *et al.*, 2016). The starch content, nutritional value, and end-use of wheat grains are all influenced by grain quality. Only a few essential genes, however, have been cloned. The NAC gene Gpc-B1 was cloned and found on 6BS, for example. It promotes nutritional remobilization, which enhances the protein, zinc, and iron content of wheat grains. Its codes for a transcription factor regulate senescence and increase wheat grains' protein, zinc, and iron content. Changes in the starch branching enzyme II gene increase amylose and resistant starch levels, suitable for human health (Slade *et al.*, 2012).

**Tolerance to abiotic stresses:** Abiotic stresses, e.g., waterlogging, drought, salt, high temperatures, chemical toxicity, and oxidative stress, cause crop loss and pose severe global threats to agriculture. Abiotic stresses often induce similar cellular harm. To maintain growth and production, plants must adapt to unfavorable conditions. Plant tolerance modification focuses on genes that protect and preserve cellular component function and structure. Current engineering techniques focus on transferring genes encoding signaling and regulatory enzymes, stress-tolerance conferring proteins, or enzymes involved in the production of functional and structural protectants (Wang *et al.*, 2003).

**Heat Stress:** Heat stress damages 58 percent of the wheat crop globally. Several model-based studies predict the increased frequency of high temperatures during the agricultural season. Wheat crops in India, China, and the United States are susceptible to short- and long-term heat stress (Tack *et al.*, 2015). According to model-based and empirical studies, increasing the temperature by 1° C lowers wheat yield by 6.4–27 percent. Heat-tolerant wheat cultivars must be developed to reduce heat stress on wheat crop output and meet future demand for wheat grain. As a result, agronomic and physiological research has been performed to understand heat stress tolerance better. The molecular basis of heat stress tolerance has also been studied (Pandey *et al.*, 2019).

**Salinity Stress:** Salinity stress impacts 800 million hectares (6%) of land worldwide, reducing wheat yields in some countries. Salinity affects 6.73 million hectares of land in India, 3.1 million hectares in Bangladesh, and 36 million hectares in China. To produce salt-tolerant wheat cultivars, we need to understand soil salinity tolerance genetics and apply it. As a result, nations such as India, Pakistan, Bangladesh, China, Egypt, and others have emphasized salt tolerance research in wheat. The environment regulates salinity tolerance, as it does heat and drought tolerance. The salt tolerance mechanism includes root  $\text{Na}^+/\text{K}^+$  absorption and transport inside the plant (Deinlein *et al.*, 2014). Salinity tolerance is age-dependent and rises with crop age. These differences exist between germination and early development QTL for salt tolerance (Yamaguchi *et al.*, 2005).

**Drought Stress:** Drought (water stress) damages 42% of the world's (218.5 million hectares) wheat-growing land, reducing agricultural yields (Kang *et al.*, 2009). According to

some estimates, rain-fed wheat (50 million hectares) receives less than 600 mm of precipitation each year, and this rainfall may be as low as 350 mm per year in places where the poorest farmers live. Sixty-six percent of the irrigated wheat crop in India is only partially irrigated, subject to water stress. Lack of irrigation water is one of the primary reasons for not cultivating wheat harvests in the North China Plains (Wang *et al.*, 2018). Drought-resistant wheat varieties are now being researched genetically worldwide. Most drought tolerance factors are complex, polygenic, and heritable (Farooq *et al.*, 2014).

**Tolerance to biotic stresses:** Scab, head blight, *Septoria tritici* Blotch (STB), etc., are fungal diseases that reduce wheat yields by about 25% annually and threaten world food security (Marchal *et al.*, 2018). Disease resistance genes in wheat cultivars have been discovered via several attempts. Rusts, such as leaf, stripe, and stem rust, are the most common and deadly wheat diseases. Lr34 is resistant to corrosion and mildew. Despite its widespread usage in wheat breeding, the Lr34 gene, which encodes an ATP-binding cassette transporter, was not cloned until 2009. The LR34 transporter, like the Arabidopsis pleiotropic drug resistance protein PEN3, confers infections by releasing chemicals that suppress fungus development. Stripe rust resistance is extensive in cloned Yr36, and it has a putative START domain and an active enzymatic (kinase) domain. Yr28, a rust-resistant NB-LRR in wheat, was recently cloned (Zhang *et al.*, 2019).

There is also resistance to Ug99 in two genes (Sr33 and Sr35) from *A. tauschii* and *Triticum monococcum* (Periyannan *et al.*, 2013). Powdery mildew can severely reduce wheat yields. Powdery mildew's resistance alleles and loci are identified and named. Only a few genes have been described molecularly and functionally. The powdery mildew resistance gene Pm21 encodes a CC-NBS-LRR protein (Xing *et al.*, 2018). At the same time, Pm60 from *T. urartu* encodes an NB-LRR protein implicated in But E09 resistance. *Staphylococcus aureus* (*Zymoseptoria tritici*) induced STB may in wheat; there are 21 Stb genes. So yet, just one gene, Stb6, has been cloned. Fighting pathogens without inducing hypersensitivity encodes conserved wall-associated receptor-like kinase (Santenac *et al.*, 2018).

**Wheat Rust:** Northern India has more significant wheat stripe rust. The most prevalent rust, *P. tritici* Eriks., damages wheat harvests globally (Roelfs *et al.*, 1992). The pathogen's ability to adapt to many climates increases the risk of crop harm. Leaf rust may cause considerable crop losses due to frequent infection cycles in a suitable environment. Infections in the early stages of development can result in 50% decreases (Huerta *et al.*, 2011).

**Karnal Bunt:** Until 1968, *Neovossia indica* Mundkur (Syn. *Tilletia indica*) was considered a minor disease. However, a severe wheat disease on the Indian subcontinent, Karnal bunt, first occurred in 1969-70. It reduces yield and degrades grain quality (Fuentes *et al.*, 1995). It is ineffectual because it is

soil, air, and seed carried. Thus, developing disease-resistant cultivars is the best way to prevent illness.

**Loose Smut:** The fungus *Ustilago tritici* (Pers.) causes loose smut, a seed-borne wheat disease. Ut1-Ut8 are resistance genes against wheat loose smut (Kassa *et al.*, 2015).

**Conclusion:** Wheat prices are anticipated to rise 1.6% a year through 2050 due to the growing population and income. As a result, worldwide wheat yields per hectare will need to increase from 3.3 to 5 tons. Because bread wheat contains an allohexaploid genome, understanding functional genomics is critical to advance inbreeding development. Identifying the most relevant genes and their structure, function, and participation in plant growth is critical to improving grain yield and quality. Using functional genomics, plant researchers may "genetically manipulate" essential gene architectures and functions. A genetic transformation is a reliable approach for proving the roles and activities of critical genes. Decades of progress have been achieved in genetic mapping tools and wheat transformation methods, with more and more wheat genes being cloned and dissected. Wheat has three sub-genomes (AABBDD) and functional redundancy in most genes. Wheat research has benefited from recent advances in genome editing, which have allowed for targeted gene alterations in all copies (i.e., not only in a single gene). This approach allows for fast wheat genetic improvement by inactivating genes that confer undesired characteristics. We now have sophisticated genome editing tools, high-quality genome sequences, bigger genetic maps, and improved transgenic technology at our disposal.

Random mutagenesis and genome editing should allow any gene mutation in the wheat genome to be detected; other approaches may then be used to determine the biological function of the mutant gene. Today's wheat selection aims to emphasize biotic stress resistance. A lack of race-specific resistance in cultivars and considerable virulence diversity in rust pathogens causes significant losses in wheat output globally.

Non-race-specific resistance genes define long-term resistance, while race-specific genes define resistance at the seedling stage. "Genomic selection" is a revolutionary technique based on the wide conventional selection using molecular markers that allows breeding programs to reduce the mean time generation interval and invest in environmentally friendly agriculture. New resistance genes might be discovered using improved genetic methods. These requirements, the qualities being studied, and the available resources should all be carefully considered.

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